**Adjuvant dendritic cell vaccination in high-risk uveal melanoma**

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**Conflict of interest**

No conflicting relationship exists for any author.Uveal melanoma (UM) is the most common primary intraocular malignancy in adults with an annual incidence of 4 to 10 per million in the Caucasian population. The 5-year overall survival (OS) rate is approximately 70-80%. Up to 50% of UM patients develop metastases, usually after a long disease-free interval (2 to 5 years). If metastatic disease is present, the prognosis is dismal with a 1-year OS rate of 10-40%. Currently, no effective systemic treatment improving OS is available for metastatic UM patients, nor has any adjuvant treatment shown survival benefit.

Our research group, as well as others, have performed several prospective dendritic cell (DC) vaccination studies in patients with cutaneous melanoma showing little toxicity and promising immunological and clinical results. DC are antigen-presenting cells with the unique capacity to activate naïve antigen-specific T cells, hence suitable to induce antitumor immune responses. The tumor antigens gp100 and tyrosinase, used in our DC vaccination studies for cutaneous melanoma patients, are both expressed in human UM tumor cells and thus constitute an appropriate target for immunotherapy in UM. Recently, we showed that DC vaccination is feasible in metastatic UM and no safety concerns were detected. Furthermore, DC vaccination showed the potential to enhance the host’s antitumor immunity, and may be associated with longer than average OS in metastatic UM.1 DC vaccination may have a more pronounced effect in the adjuvant setting as high tumor burden in metastatic patients may hamper the induction of effective immune responses. Preferably, patients with a high risk for development of metastatic disease are selected for adjuvant treatment. In primary UM, monosomy 3 correlates strongly with the development of metastases and decreased survival (3-year OS rate 60% with monosomy 3 versus 95-100% with disomy 3) and is identified in approximately 50% of patients.2

Therefore, we performed an open label phase II study in high-risk UM patients with monosomy 3, investigating immunological responses after adjuvant DC vaccination. Inclusion criteria included HLA-A\*02:01-positivity, interval since local treatment<12 months, and age 18-75 years. Patients with distant metastases were excluded. Patients received autologous, monocyte-derived DC transfected with mRNA encoding the tumor-antigens gp100 and tyrosinase according to a schedule of 3 biweekly intradermal and intravenous vaccinations. In the absence of disease recurrence, patients received a maximum of two maintenance cycles at 6-month intervals. Ethics Committee approval was obtained and written informed consent was obtained from all patients (NCT00929019). Due to low accrual rates, mainly caused by the rarity of the tumor, older age at diagnosis, HLA restriction, and the increase of eye-conserving treatments interfering with the availability of tumor material for genetic testing, the trial was stopped prematurely. Still, 23 patients received at least one cycle of adjuvant DC vaccination and were considered evaluable; 18 patients completed all 3 cycles of vaccinations. Baseline characteristics are shown in Supplementary Table 1 (available at http://aaojournal.org).. DC vaccinations were well tolerated. Side effects associated with DC vaccination were transient flu-like symptoms in 91% of patients, and erythema at the site of injection in 87% of patients. Vitiligo occurred in one patient. No treatment related grade 3 or 4 toxicity was observed.

To test the capacity of the patients in this study to generate an immune response upon vaccination, DC were loaded with keyhole limpet hemocyanin (KLH), a control antigen. All patients tested showed a cellular response to KLH, indicating that the vaccine induced de novo immune responses. Previously, we showed that the presence of tumor-specific T cells in cultures of skin-test infiltrating lymphocytes positively correlated with clinical outcome in metastatic cutaneous melanoma patients. Therefore, skin tests were performed after each vaccination cycle and the presence and functionality of tumor-specific T cells induced by DC vaccination was analyzed. Tumor-specific T cells in the skin tests were present in 17 patients (74%), demonstrating the effectiveness of these type of vaccines. Our previous findings in metastatic UM patients showed a lower tumor-specific immunologic response rate, as only 29% of metastatic UM patients showed tumor-specific CD8+ T cells after DC vaccination. Even if we only take the first skin test into account, the difference remains remarkable. We observed similar differences in the rates of cutaneous melanoma patients in the metastatic and adjuvant setting.3 Therefore, the hypothesis that DC vaccination might be more potent in the adjuvant setting is further supported by this study.

Up to April 2016, 9 patients (39%) are free of melanoma relapse and 14 patients (61%) developed metastatic disease after DC vaccination of which 12 patients have died (52%). The median disease-free survival (DFS) was 34.5 months (95%CI, 27.2-41.8 months), with a 3-year DFS rate of 47%. The median OS was 51.8 months (95%CI, 42.1-62.7 months), with a 3-year OS rate of 79%. When patients were analyzed separately, based on the presence or absence of tumor-specific T cells in the skin test, patients with tumor-specific T cells had better DFS and OS. No large differences were seen in their baseline characteristics (Supplementary Table 1; available at http://aaojournal.org). In patients with tumor-specific T cells after DC vaccination median DFS was 51.9 vs 18.8 months in patients in whom we could not detect tumor-specific T cells (*p*=0.024; Figure 1A). Median OS was 45.0 months with a 3-year OS rate of 60% for patients without detectable tumor-specific T cells and 58.0 months and 87% for patients in whom tumor-specific T cells were found (*p*=0.016; Figure 1B).

Taking the restrictions of comparing results of small studies with historical data into account, the 3-year OS rate of the DC vaccinated patients (79%) compared well to literature (approximately 60%in high-risk UM).2, 4 In theory, HLA-A\*02:01 phenotype could be a confounding factor, but no correlation with survival is shown in a large cohort of UM patients.5 Of course, a randomized trial is needed to provide a definitive conclusion on the effect of DC vaccination in high-risk UM patients, which is currently opened elsewhere (NCT01983748).

In conclusion, adjuvant treatment with DC vaccination in high-risk UM patients gives little toxicity and correlates with favorable OS in patients with a detectable tumor antigen-specific immune response after DC vaccination. Further evidence for the clinical efficacy of adjuvant DC vaccination should be obtained from prospective randomized clinical trials.

**Figure legends**

**Figure 1. Survival in correlation with the presence of tumor antigen-specific T cells after adjuvant DC vaccination.** Kaplan-Meier curves of disease-free survival (**A**) and overall survival (**B**) for patients with high-risk UM who received adjuvant DC vaccination after treatment of the primary tumor according to the presence (Tc+; n=17; solid black line) or absence (Tc-; n=6; dashed grey line) of tumor antigen-specific T cells in skin-test infiltrating lymphocytes. Survival was calculated from calculated from the treatment of the primary tumor. Statistical significance was determined by a log rank test.

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**Supplementary Table 1. Baseline characteristics.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **All patients n=23** | **%** | **T cell positivea n=17** | **%** | **T cell negativeb n=6** | **%** |
| **Sex** |  |  |  |  |  |  |
| Male | 12 | 52% | 10 | 59% | 2 | 33% |
| Female | 11 | 48% | 7 | 41% | 4 | 67% |
| **Age, years** |  |  |  |  |  |  |
| Mean (range) | 56 (31-69) |  | 56 (31-69) |  | 54 (36-69) |  |
| **T stage** |  |  |  |  |  |  |
| T1 | 1 | 4% | 1 | 6% | 0 | 0% |
| T2 | 9 | 39% | 7 | 41% | 2 | 33% |
| T3 | 12 | 52% | 9 | 53% | 3 | 50% |
| T4 | 1 | 4% | 0 | 0% | 1 | 17% |
| **T location** |  |  |  |  |  |  |
| Choroid | 15 | 65% | 12 | 71% | 3 | 50% |
| Ciliary body + Choroid | 8 | 35% | 5 | 29% | 3 | 50% |
| **T histology** |  |  |  |  |  |  |
| Mixed | 14 | 61% | 10 | 59% | 4 | 67% |
| Epitheloid | 3 | 13% | 2 | 12% | 1 | 17% |
| Spindle | 3 | 13% | 2 | 12% | 1 | 17% |
| Unknown | 3 | 13% | 3 | 18% | 0 | 0% |
| **T tumor size mean (range)** |  |  |  |  |  |  |
| Diameter (mm) | 14 (7-23) |  | 7 (2-12) |  | 9 (4-12) |  |
| Thickness (mm) | 7 (2-12) |  | 14 (7-18) |  | 13.5 (10-23) |  |
| **Local treatment** |  |  |  |  |  |  |
| Enucleation | 18 | 78% | 12 | 71% | 6 | 100% |
| Stereotactic radiotherapy | 4 | 17% | 4 | 24% | 0 | 0% |
| Plaque brachytherapy | 1 | 4% | 1 | 6% | 0 | 0% |

**a** presence of tumor antigen-associated specific T cells in the skin test.

b absence of tumor antigen-associated specific T cells in the skin test**.**

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